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Antibodies against cyclic citrullinated peptide in rheumatoid arthritis patients: relation to disease duration and activity

Rheumatoid arthritis (RA) is a systemic, autoimmune disease, characterized by chronic, synovial inflammation and autoantibody production. A broad range of autoantibodies has been found in RA patients. The first detected and best known antibodies are rheumatoid factors (RFs). The presence of IgM-RF is one of the classification criteria for RA by American College of Rheumatology (ACR) (2). RFs are detected in 70–80% of patients with established RA, but can also be present in patients with other autoimmune diseases or chronic infections and even in 3–5% of healthy subjects, particularly in the elderly (15).

Another family of autoantibodies to citrullinated proteins has lately been shown to be more specific for RA (antiperinuclear factor (APF), antikeratin (AKA), anti-Sa and antifilaggrin antibodies (AFA) (6). These are the so-called anti-citrullinated protein (anti-CCP) antibodies. Citrulline is a non-coded native amino acid. Citrulline-containing peptides are only produced in the process of posttranslational modification, by enzymatic conversion of peptidylarginine to citrulline. The enzymes responsible for the conversion are peptidylarginine deiminases (PADIs) and two of them (PADI2 and PADI4) have been found in the RA synovium (11). Autoimmunity against citrulline-containing self-peptides is highly specific for RA and useful in early diagnosis. There have been assays detecting anti-CCP antibodies developed recently. The second generation test (CCP2) using highly reactive peptides is extremely specific for RA (98%), and has sensitivity similar to the IgM-RF (68- 80%) (15). Recent studies have reported, that anti-CCP antibodies could be detected very early in the course of the disease, even preceding the clinical symptoms of RA (12, 13). Nielen et al. detected anti-CCP1 antibodies in sera of blood donors up to 14 years before the first clinical manifestation of RA (12). In another study Rantapää-Dahlqvist et al. detected anti-CCP2 antibodies up to 9 years before clinical presentation, with the frequency of positive samples increasing close to the disease onset (13). Other studies indicate that anti-CCP antibodies are able to differentiate RA from undifferentiated polyarthritis in patients with early arthritis (14). Prognostic value of anti-CCP antibodies has been investigated in groups of patients with early RA indicating that anti-CCP positivity was correlated with more aggressive course of the disease and more severe radiographic damage (7, 15).

The aim of this study was to compare chosen clinical and biochemical parameters between the following groups of RA patients: anti-CCP positive and anti-CCP negative ones.

MATERIAL AND METHODS

The study group consisted of 74 RA patients (pts) treated in the Department of Rheumatology and Connective Tissue Diseases, Medical University of Lublin, and in the out-patient clinic, between January 2004 and November 2004. All pts fulfilled the 1987 ACR criteria for RA (2) and were treated with disease-modifying antirheumatic drugs (DMARDs). Patients' charts were reviewed for demographic information, clinical diagnosis, radiographic information. Clinical and laboratory data were collected and used to determine the disease activity score; using 28 joints score (DAS28). Serum samples were obtained and stored at –80°C. Serum anti-CCP antibodies were analyzed by commercial enzyme linked immunosorbent assay (ELISA; Immunoscan RA, Euro-Diagnostica). Serum samples with a test result greater than 50 units/ml were considered positive. Serum levels of C-reactive protein (CRP) were measured by immunoturbidimetric assay, with the upper limit of the normal range at 0.5 mg/l. Serum cystatin C was measured using quantitative enhanced immunonephelometry method (with commercially available assay developed by DADE Behring). The producer recommended the normal range of cystatin C between 0.53 and 0.95 mg/dl, independently of sex, age and body mass. Serum albumin was measured by photometric test with bromocresol green (normal range 3.8–5.1 g/dl). We also determined body mass index (BMI) in every patient.

Statistics. The χ^2 test (with Yate's correction when appropriate) was used for qualitative parameters. Correlation between quantitative variables was assessed by Spearman's correlation coefficients. In order to compare subgroups of patients who were positive or negative for anti-CCP antibodies, Student's test or non-parametric Mann-Whitney *U* test, respectively, were used. For all tests, *P* values less or equal to 0,05 were considered significant.

RESULTS

Patient characteristics. The study group consisted of 74 RA pts: 51women (68.9%) and 23 men (31.1%). Their mean (SD) age was 57.96 (14.17) years and the mean (SD) disease duration was 120.8 (100.6) months (range 3 to 480 months). Long-standing RA (defined by disease duration ≥ 36 months) was observed in 60 pts (81.1%). RF was present in 48 pts (64.9%). Bone erosions were observed in 52 pts (70.3%) and extra-articular manifestations of RA in 23 pts (31.1%). The mean (SD) DAS28 was 4.99 (1.79) with range 1.36 to 8.29. High disease activity, defined as DAS28 ≥ 5.1 was noticed in 36 pts (48.6%); moderate disease activity (DAS28 ≥ 3.2 and < 5.1) was observed in 19 pts (25.7%). The mean (SD) number of tender joints was 9.68 (8.55) (range 0 to 28 joints) and swollen joints 6.47 (6.15) (range 0 to 25 joints). The mean (SD) serum CRP was 32.5 mg/l (35.1) (range 1.7–146.8). The mean (SD) serum albumin was 3.84 g/dl (0.53) (range 1.56–4.71). The mean (SD) hemoglobin concentration was 12.37 g/dl (1.69) (range 7.58–17.0). The mean (SD) serum creatinine was 0.92 mg/dl (0.22) (range 0.31–1.63) and serum cystatin C 1.21 mg/l (0.45) (range 0.7–2.53). The mean (SD) BMI was 25.49 (3.95) (range 17.31–34.05).

Comparison of anti-CCP positive and anti-CCP negative pts. Serum samples from 55 pts (74.3%) were anti-CCP positive (group 1). Nineteen pts (25.7%) were negative for anti-CCP antibodies (group 2). The mean (SD) value of the anti-CCP antibodies titer was 903.55 (1,356.8) (range 50.1 to 5,380) in positive sera. Clinical and biochemical parameters of both groups of patients are summarized in Table 1. Patients in group 1 were significantly older than group 2 pts. Other demographic factors (gender, disease duration) were not significantly different between the groups. Neither the presence of RF nor joint erosions were associated with the presence of anti-CCP antibodies. Extra-articular manifestations occurred more often in group 1 pts, but the difference did not reach statistical significance.

Table 1. Comparison of anti-CCP positive (group 1) and anti-CCP negative (group 2) pts

Variable	Group 1 (55 pts)	Group 2 (19 pts)	pValue
Gender (F/M)	38 (69.1%)/ 17(30.1%)	13 (68.4%)/ 6 (31.6%)	>0.1
Age (years)	60.1 (12.6)	51.8 (16.9)	0.03*
Disease duration (months)	124.4 (104.9)	110.3 (88.5)	>0.1
Long-standing RA	44 (80%)	16 (84.2%)	>0.1
DAS28 ≥ 3.2	44 (80%)	11 (67.9%)	0.06
Extra-articular symptoms	20 (36.4%)	3 (15.8%)	>0.1
RF positive	37 (67.3%)	11 (57.9%)	>0.1
Erosions	39 (70.9%)	13 (68.4%)	>0.1
ESR (mm/h)	42.2 (27.5)	33.6 (23.7)	>0.1
CRP (mg/l)	33.1 (34.6)	30.6 (37.6)	>0.1
Number of tender joints	10.8 (8.7)	6.3 (7.4)	0.03*
Number of swollen joints	7.2 (6.2)	4.4 (5.5)	0.04*
DAS28 (0- 10)	5.2 (1.7)	4.3 (1.9)	0.04*
Serum albumin (g/dl)	3.75 (0.55)	4.09 (0.35)	0.02*
Hemoglobin (g/dl)	12.15 (1.6)	12.97 (1.9)	0.07
BMI (kg/m^2)	25.4 (3.9)	25.6 (4.3)	>0.1
Serum creatinine (mg/dl)	0.9 (0.21)	0.97 (0.24)	>0.1
Serum cystatin C (mg/dl)	1.28 (0.46)	1.03 (0.39)	0.09

Values are: mean (SD) or n (%) * Statistically significant

Table 2. Characteristics of anti-CCP positive (group 1a) and anti-CCP negative (group 2a) pts with disease duration ≥ 36 months

Variable	Group 1a (44 pts)	Group 2a (16 pts)	p value
Gender (F/M)	31 (70.5%)/ 13(29.5%)	11 (68.8%)/ 5 (31.2%)	>0.1
Age (years)	60.9 (12.3)	52.8 (12.6)	0.03*
Disease duration (months)	151.3 (100.5)	127.6 (85.8)	>0.1
DAS28 ≥3.2	35 (79.5%)	10 (62.5%)	0.08
Extra-articular symptoms	19 (43.2%)	3 (18.8%)	>0.1
Rheumatoid factor positive	32 (72.7%)	9 (56.3%)	>0.1
Erosions	37 (84.1%)	12 (75.0%)	>0.1
ESR (mm/h)	41.9 (26.6)	34.0 (22.3)	>0.1
CRP (mg/l)	34.4 (34.9)	29.9 (36.9)	>0.1
Number of tender joints	10.9 (8.6)	6.8 (7.6)	0.07
Number of swollen joints	7.2 (6.3)	4.5 (5.9)	0.05*
DAS28 (0- 10)	5.3 (1.7)	4.4 (1.8)	0.08
Serum albumin (g/dl)	3.68 (0.57)	4.1 (0.34)	0.01*
Hemoglobin (g/dl)	12.14 (1.6)	13.07 (1.8)	0.06
BMI (kg/m^2)	25.4 (4.1)	26.2 (4.4)	>0.1
Serum creatinine (mg/dl)	0.89 (0.21)	0.99 (0.26)	>0.1
Serum cystatin C (mg/dl)	1.31 (0.37)	1.06 (0.24)	0.04*

Values are: mean (SD) or n (%) *Statistically significant

High and moderate disease activity (DAS28 ≥ 3.2) was associated with anti-CCP positivity, but it was not statistically significant. The mean DAS28 value was significantly higher in group 1 pts than in group 2 pts. The numbers of tender joints and swollen joints were also significantly greater in group 1 pts. Mean serum albumin was significantly lower in group 1 than in group 2 pts. BMI value was similar in both groups. However, only in group 1 there were 4 pts severely malnourished with albumin level < 3 g/dl and BMI < 20 kg/m^2. Mean hemoglobin concentration was not significantly

lower in group 1 than in group 2 pts. Mean values of CRP, erythrocyte sedimentation rate (ESR), serum creatinine and cystatin C did not differ significantly between the two groups.

Comparison of anti-CCP positive and anti-CCP negative pts with long-standing RA. When we take into consideration pts with long-standing RA: 44 pts (73.3%) were anti-CCP positive (group 1a) and 16 pts (26.7%) were anti-CCP negative (group 2a). Characteristics of these patients are presented in Table 2. Patients in group 1a were significantly older than in group 2a. Gender and disease duration were similar in the two groups. The presence of RF and extra-articular manifestations were observed more often in group 1a, but differences were not statistically significant. There were significantly more swollen joints in group 1a than in group 2a. Mean serum albumin was significantly lower in group 1a. Mean serum cystatin C was significantly higher in group 1a and we found a significant positive correlation between serum creatinine levels and the levels of anti-CCP antibodies (r_s = 0.303; p= 0.045). Mean hemoglobin concentration was lower in group 1a. The number of tender joints, DAS28, ESR and CRP concentration did not differ significantly between the compared groups.

Differences between anti-CCP positive pts with long and short RA duration. The group of anti-CCP positive pts consisted of 44 pts (80%) with long-standing RA (1a) and 11 pts (20%) with disease duration <36 months (1b). Comparison between the two groups (1a vs 1b) revealed statistically significant differences in: mean serum albumin (3.68 ± 0.57 vs 4.04 ± 0.38; p=0.03), number of patients with bone erosions (37 (84.1%) vs 2 (18.2%); p=0.002), and number of patients with extra-articular manifestations (19 (43.2%) vs 1 (9.1%); p=0.04). Other demographic features, levels of anti-CCP antibodies and parameters of disease activity did not differ significantly between the groups.

DISCUSSION

In our study group of 74 pts with RA, anti-CCP antibodies were present in 55 pts (74.3%). In the literature the prevalence of these antibodies varies in different study populations: from 55% (7) and 58.9% (11) in pts with recent RA onset, to 83% (4) and 88% (1) in pts with aggressive RA, who were selected for anti-TNFα (infliximab) treatment, suggesting the association between the presence of anti-CCP antibodies and severity of the disease. The majority of our pts suffered from long-standing, active form of RA and the prevalence of anti-CCP in this group was high.

In the present study we observed a significant association between anti-CCP positivity and the number of tender joints, the number of swollen joints and the value of DAS28. Disease activity defined by these parameters was significantly higher in anti-CCP positive pts. In pts with long-standing RA there was a significant association between the number of swollen joints and the presence of anti-CCP antibodies, as well as the tendency of higher DAS28 and greater number of tender joints in anti-CCP positive pts. This suggests that the presence of anti-CCP antibodies is associated with higher disease activity, either in recent onset or in long-standing RA.

In the literature there are few reports describing an association between anti-CCP antibodies and disease activity. Forslind et al. (7) have evaluated the prevalence of anti-CCP antibodies in pts with early RA and after a 2 years' follow-up. They have reported that when comparing anti-CCP positive and anti-CCP negative pts, at the end point the anti-CCP positive pts had higher DAS28, higher CRP and ESR. Recently, studies assessing clinical and laboratory response to infliximab treatment (1, 4) and changes following B lymphocyte depletion therapy (5) have been presented. Alessandri et al. (1) observed a significant decrease in the titre of anti-CCP antibodies and RF after 6 months of infliximab treatment, but only in pts with clinical improvement. However, no pts who

were positive for anti-CCP or RF at baseline became negative at the end of the study. They also showed that variation in anti-CCP was positively correlated with variation in tender joint count. Bobbio-Pallavicini et al. (4) reported that after 30 weeks of infliximab treatment anti-CCP antibody titres significantly decreased, but returned to baseline thereafter. Cambridge et al. (5) observed a positive clinical response following B lymphocyte depletion therapy and it was correlated with a significant drop in the levels of CRP, RF and anti-CCP antibodies. Kastbom et al. (8) concluded that anti-CCP antibody positivity at diagnosis predicted higher disease activity over the following 3 years of recent onset RA. In anti-CCP positive cases they observed consistently higher ESR, CRP levels and physician's global assessment of disease activity.

In our study anti-CCP positive pts were significantly older than anti-CCP negative. The prevalence of anti-CCP antibodies in elderly pts should not be connected only with process of ageing, but rather with activity of the disease, even in long-standing RA. In the literature, Bas et al. (3) estimated the presence of anti-CCP antibodies in three populations: RA pts, non-RA controls with rheumatic diseases and in healthy blood donors. Interestingly, anti-CCP antibodies were detected in 2% of healthy blood donors, but not in elderly donors (≥ 60 years of age).

The results of our study showed for the first time an association of anti-CCP positivity with lower serum albumin, hemoglobin concentrations and BMI values. Serum albumin level, one of the approved markers of nutritional status is usually decreased in chronic inflammation. Malnutrition defined by low serum albumin and BMI value is an important risk factor of cardiovascular mortality in RA pts (9). Our results show that anti-CCP positive pts have a greater risk of protein and caloric malnutrition connected with the disease activity.

In our group of pts the presence of anti-CCP antibodies correlated also with parameters of glomerular filtration. Serum cystatin C is a parameter claimed to be superior to serum creatinine in estimating glomerular filtration rate (10). Serum cystatin C was significantly higher in anti-CCP positive pts with long-standing RA. We have also found a positive correlation between serum creatinine levels and the levels of anti-CCP antibodies in these pts. The results show that anti-CCP positive pts face a greater risk of renal insufficiency as a result of active, chronic disease. In our pts the presence of RF and extra-articular manifestations was observed more often in anti-CCP positive cases, but differences did not reach a statistical significance. The literature concerning the association of RF and anti-CCP antibodies is controversial, supporting our observations (13) or reporting higher frequency of RF in anti-CCP positive pts (7). In the literature, no association between anti-CCP antibodies and extra-articular manifestations was observed by De Rycke et al. (6).

Anti-CCP positivity in our pts was also not associated with bone erosions. In our study the presence of erosions, but not radiological progression was assessed. Recently De Rycke et al. (6) described a significantly greater "radiological progression rate" (modified Larsen score divided by the disease duration in years) in anti-CCP positive pts with long-standing RA (6). Previous studies demonstrated that anti-CCP antibodies were associated with radiological joint destruction as measured by Larsen score (7), when regarding early RA (disease duration one year or less). The presence of anti-CCP antibodies at RA onset is considered as an independent predictor of radiological damage and progression (7). However, not all the pts, who develop early erosive disease are positive for anti-CCP, that is why combined assessment of anti-CCP and IgM RF is recommended to predict a more progressive disease. Van Gaalen et al. (15) suggested that concomitant presence of anti-CCP antibodies and HLA class II RA susceptibility alleles is indicative of severe disease course and joint damage.

CONCLUSIONS

It has been recommended to analyse anti-CCP antibodies when evaluating patients with early arthritis, because of their very high specificity for RA. Anti-CCP antibodies could also be considered as a parameter in monitoring the clinical course of RA. Data from literature and our results suggest that anti-CCP antibodies are associated with RA activity, degree of renal impairment and malnutrition in the course of the disease.

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SUMMARY

Autoantibodies directed against cyclic citrullinated peptide (anti-CCP) are considered to be a highly specific marker of rheumatoid arthritis (RA). The aim of the study was to investigate the frequency of prevalence and the association of anti-CCP antibodies presence with RA duration and activity. Seventy-four patients (pts) with RA were included in the study. Long-standing RA (duration ≥ 36 months) was noted in 60 pts (81.1%). Disease activity score (DAS28) was determined. Anti-CCP antibodies, markers of nutritional status (serum albumin, BMI) and renal function (serum creatinine and cystatin C) and were assessed in all pts. Serum samples from 55 pts (74.3%) were anti-CCP positive (group 1), and from 19 patients (25.7%) were anti-CCP negative (group 2). Comparison of the two groups revealed significant differences: higher mean value of DAS28, greater number of tender and swollen joints, but lower mean serum albumin in pts from group 1. The proportion of pts with long-standing RA was similar in both study groups (80.0% in group 1 and 84.2% in group 2). In long-standing RA, anti-CCP positive pts (group 1a) were characterized by a significantly higher number of swollen joints, lower serum albumin and higher serum cystatin C, compared with long-standing RA anti-CCP negative pts (group 2a). Our results suggest that the presence of anti-CCP antibodies is associated with RA activity, as well as the risk of malnutrition and renal impairment in the course of the disease.

Przeciwciała przeciwko cyklicznemu peptydowi cytrulinowemu u chorych na reumatoidalne zapalenie stawów: związek z aktywnością i czasem trwania choroby

Przeciwciała przeciwko cyklicznemu peptydowi cytrulinowemu (anty-CCP) są uznawane za wysoce specyficzny marker reumatoidalnego zapalenia stawów (RZS). Celem naszej pracy było zbadanie częstości występowania przeciwciał anty-CCP u chorych na RZS oraz zależności między obecnością tych przeciwciał i czasem trwania i aktywnością choroby. W badanej grupie było 74 chorych na RZS. Długotrwały przebieg choroby (czas trwania RZS ≥ 36 miesięcy) zanotowano u 60 chorych (81,1%). U wszystkich chorych określono: wskaźnik aktywności choroby (DAS28), poziom przeciwciał anty-CCP, parametry stanu odżywienia (stężenie albuminy w surowicy, BMI) i funkcji nerek (stężenie kreatyniny i cystatyny C w surowicy). Obecność przeciwciał anty-CCP stwierdzono u 55 chorych (74,3%) (grupa 1), u 19 (25,7%) nie stwierdzono ich obecności (grupa 2). Porównując obie badane grupy, zaobserwowano różnice istotne statystycznie: wyższą średnią wartość DAS28, większą liczbę stawów bolesnych i obrzękniętych oraz niższe stężenie albuminy w surowicy u chorych z grupy 1. Odsetki chorych z długotrwałym RZS były porównywalne w obu grupach (80,0% w grupie 1 i 84,2% w grupie 2). W grupie chorych z długotrwałym RZS i obecnymi przeciwciałami anty-CCP (grupa 1a) stwierdzono: istotnie statystycznie większą liczbę stawów obrzękniętych, niższe stężenie albuminy w surowicy oraz wyższe stężenie cystatyny C w surowicy w porównaniu z grupą chorych z długotrwałym RZS bez obecności przeciwciał anty-CCP (grupa 2a). Wyniki naszej pracy wskazują na to, że obecność przeciwciał anty-CCP u chorych na RZS jest związana z wyższą aktywnością choroby, jak również z ryzykiem niedożywienia i upośledzenia funkcji nerek w przebiegu choroby.